Stromal scoring in advanced colon and rectal cancer: stroma-rich tumors and their association with aggressive phenotypes

Ricella M. Souza da Silva[1,2], Eduardo M. Queiroga[3], Cynthia A. B. de Toledo Osório[4], Karin S. Cunha[2] and Eliane P. Dias[5]

SUMMARY

Background: *Our aim was to explore relevance of the proportion between neoplastic cell component and tumor-associated stroma in order to assess its association with confirmed aggressive phenotypes of right/left colon and rectum cancers in a large series of patients.* **Methods:** *The quantification of stroma component was performed in patients diagnosed with colorectal adenocarcinoma who underwent surgical resection. The analyzed variables were age, gender, anatomical/pathological features, and tumor-stroma proportion. Tumor-stroma proportion was estimated based on slides used in routine pathology for determination of T status and was described as low, with a stromal percentage ≤50% or high, with a stromal percentage >50%. The tumor-stroma proportion was estimated by two observers, and the inter-observer agreement was assessed.* **Results:** *The sample included 390 colorectal adenocarcinoma patients. Stroma-rich tumors were observed in 53.3% of cases. Well-differentiated tumors had the lowest stromal proportions ($p = 0.028$). Stroma-poor tumors showed less depth of invasion ($p<0.001$). High stromal content was observed in association with tumor budding, perineural, angiolymphatic, and lymph node involvement, and distant metastasis ($p\leq0.001$). Colorectal adenocarcinoma without lymph node or distant metastasis involvement had lower stromal proportion, while metastatic ones exhibited high stromal content ($p <0.001$). The inter-rater reliability (concordance) between the estimations of pathologists for tumor-stroma proportions was high ($\kappa=0.746$).* **Conclusion:** *The tumor-stroma proportion in colorectal adenocarcinoma was associated with adverse prognostic factors, reflecting the stage of the disease. Stroma-rich tumors showed a significant correlation with advancement of the disease and its aggressiveness. Due to its availability tumor-stroma proportion evaluation has high application potential and can complement current staging system for colorectal adenocarcinoma.*

Keywords: *Tumor microenvironment; Tumor-stroma proportion; colorectal cancer; advanced disease; aggressive phenotype*

INTRODUCTION

Cancer is currently recognized as a complex disease composed of several cell types, especially those derived from the surrounding mesenchymal stroma, with which neoplastic cells establish the tumor microenvironment (TME). In this environment, the tumor stroma represents one of the TME components (1–4). Tumor cells explore their stroma, changing its composition in a bi-directional communication, leading to the stromatogenesis. The interaction pathways are varied and complex. Therefore, the stromal tissue is not a passive component that involves the tumor (5).

Studies have shown that tumor stroma plays a relevant and diverse role in tumorigenesis, acting in different stages: it facilitates the survival and proliferation of neoplastic cells; promotes epithelial-mesenchymal transition, and local and metastatic spread (6–12). Even in distant and lymph node metastatic sites, stromal components accompany cancer cells (13, 14).

In malignant epithelial tumors, the scoring system based on the evaluation of the tumor-stroma proportion (TSP) in sections stained with hematoxylin and eosin (H&E) has been shown to be a good prognostic tool (9,10,15–18). Several international research groups have demonstrated that high amount of stroma contributes to a more aggressive tumor phenotypes (8, 9, 15–17, 19–21). Their goal was to fill the need for and identify new prognostic characteristics that could be used along with the current pathological staging (8, 20).

The traditional tumor, lymph node and metastasis (TNM) system that has been used routinely for prognosis estimate and guidance of treatment for certain types of tumors (22, 23), lacks accuracy (21, 24, 25). In colorectal cancer (CRC), new reliable biomarkers are needed to guide personalized treatment (21) since current pathological variables only moderately indicate possible outcome and response to therapy (6, 19, 21). Currently, CRC represents a serious public health problem worldwide, occupying the third place in terms of incidence and the second place in mortality numbers (22), while being little attended by public policies in underdeveloped or developing countries (26).

Although the complete biological role of stroma is not yet fully understood (20), in the last 10 years the evaluation of tumor stroma has gained interest due to its simplicity and, above all, to its clinical value as a potential prognostic factor. Furthermore, cancer cells and stroma are being considered as therapeutic targets in treatment strategies for solid tumors (14), in which the quantification of the stromal component may provide additional risk stratification for adaptation to neoadjuvant and adjuvant treatments (8, 15, 20, 27).

The aim of this study was to evaluate the relevance of the tumor-stroma proportion and its association with confirmed aggressive phenotypes in a large series of patients diagnosed with cancer of the right/left colon and rectum in Brazil.

METHODS

Tissue collection consisted of samples from colorectal adenocarcinoma (CRA) patients treated with curative surgery from year 2013 to 2018.

Arch Oncol 2022; 28(1):1-6

Published Online
June 30th, 2021
https://doi.org/10.2298/AOO2104003003S

[1] Pathological Anatomy Service, Lauro Wanderley University Hospital of Federal University of Paraíba, João Pessoa, Paraíba, 58050-585, Brazil

[2] Postgraduate Program in Pathology, Fluminense Federal University, Niterói, Rio de Janeiro, 24033-900, Brazil

[3] Laboratory of Pathological Anatomy, Alcides Carneiro University Hospital of the Federal University of Campina Grande, Campina Grande, Paraíba, 58400-398, Brazil

[4] Department of Pathology, A. C. Camargo Cancer Center, São Paulo, 01525-001, Brazil

[5] Department of Pathology, Fluminense Federal University, Niterói, Rio de Janeiro, 24033-900, Brazil

Correspondence to:
Ricella M. Souza da Silva
ricellasouza@gmail.com
ORCID: https://orcid.org/0000-0002-3860-6660

Received **2021-04-03**
Received in revised form **2021-06-14**
Accepted **2021-06-14**

Patients that received neoadjuvant treatment with radiotherapy and/or chemotherapy were excluded from the study. The study was approved by the local Ethics Committee (registration No.: 03283218.6.0000.5183) of the Lauro Wanderley University Hospital of the Federal University of Paraíba, Brazil.

Histopathological data were obtained from the re-analysis of the histological slides of the surgical specimens and from the respective anatomical/pathological report. Clinical data were obtained from the medical and hospital records. Evaluated clinicopathological data included age, gender, tumor topography, histological type, tumor grade (differentiation), depth of invasion, tumor budding, perineural invasion, angiolymphatic invasion, lymph node involvement, and presence of distant metastasis according to the Union Internationale Contre le Cancer / American Joint Cancer Committee (UICC/AJCC) TNM staging system (22, 23).

Tissue samples consisted of 4 μm thick, hematoxylin and eosin stained histological sections. The deepest invasive part of the bowel wall of the surgical specimen of primary tumor (slides used in routine pathology to determine pT-category) was used to evaluate the stromal proportion. Areas with the largest amount of stroma were selected by conventional microscopy using 2- 5 × objective magnification. Subsequently, an area containing both tumor and stromal tissue was selected using 10 × objective magnification. Tumor cells were present at all edges of the selected image field (north-east-south-west) for quantification of the stromal component. Areas with large amount of muscle, mucus, necrosis or large vessels, as well as tears or tissue retraction artefacts were not included. Infiltration with inflammatory cells is not an exclusion criterion (28) and it was included in the scoring (Figure 1). When there was more than one area with the high number of stroma visible, the area with the highest percentage of stroma was selected. In the case of only one doubtful area / field of view categorized as high stroma (even after consulting a second observer), the total composition of the entire tissue section was evaluated with the objective magnifications of 2 - to 5 × for classification of that particular case (28).

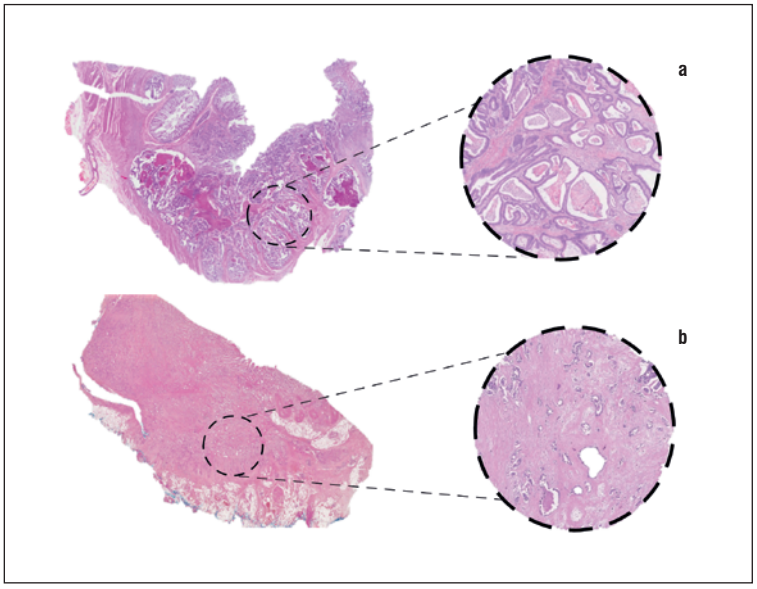

Figure 1. Standardized scoring of stromal estimation in colorectal adenocarcinoma. Selection of the area with the highest amount of stroma with tumor cells present at all borders of the image field: a) Case No. 190, b) Case No. 291. Hematoxylin-eosin; magnifications ×2 and ×10.

Statistical Analisys

Scoring percentages were semi-quantitatively reported as tenfold percents (10%, 20%, 30%, etc.). For statistical analyses and for the comparison with clinicopathological data samples were grouped as stroma-high (TSP >50%) and stroma-low (TSP ≤50%) as previously described (4, 9, 10, 15–17).

Statistical analyses were performed using Statistical Package for the Social Sciences, Version 20 (SPSS Inc., Chicago, IL). Data were expressed as absolute and relative values. A descriptive analysis of the variables was performed. Likelihood ratio test was used to investigate the effect of the variables on the stromal proportion. The level of significance was set at 0.05.

The stromal scoring was estimated by two observers (RMSS and EMQ). The inter-observer agreement for TSP assessment, reported as categorical data, was determined using Kappa (κ) concordance index and the intra-class correlation coefficient (ICC). These criteria categorize a score of 0 as poor, 0 to 0.2 slight, 0.2 to 0.4 fair, 0.4 to 0.6 moderate, 0.6 to 0.8 substantial, and 0.8 to 1.0 almost perfect (29).

RESULTS

Tissue collection Tissue collection consisted of 390 cases of CRA. The mean age was 63.5 years, with 81.8% of individuals over 50 years old and 6.4% of patients under 40 years old. The clinicopathological data were presented in Table 1.

The tumor-stroma proportion was evaluated in 384 cases. In six cases, analisis could not be performed due to the absence of a deeper histological slide or due to insufficient size of tumor tissue. Stroma-rich tumors (stromal estimate above 50%) were observed in 53.3% of the cases (Figure 2).

Well-differentiated tumors had the lowest stromal proportion ($p = 0.028$). Stroma- poor tumors showed less depth of invasion ($p < 0.001$). High stromal content was observed when perineural invasion, angiolymphatic invasion, lymph node involvement, distant metastasis and tumor budding occurred ($p \leq 0.001$).

CRA p-Stage I (T1-T2, N0, M0) and p-Stage II (pT3-T4, pN0, pM0) were predominantly poor in stroma (estimated stroma ≤ 50% : 81.4% and 51.6%, respectively), while 65% of CRA p-Stage III (any pT, pN1-2, pM0) and 80.6% of CRA p-Stage IV (any T, any N, M1) exhibited high stromal content ($p < 0.001$) (Table 2).

There were no statistically significant associations between the stromal proportion and sex ($p = 0.952$), age groups ($p = 0.992$), tumor location ($p = 0.386$), or histological type ($p = 0.895$).

The concordance between the TSP percentages of two pathologists was substantial, with $\kappa > 0.6$ ($\kappa = 0.746$). The ICC values were above 0.8. ICC for consistency was 0.877 (0.816-0.917) and ICC for agreement was 0.823 (0.471-0.919).

DISCUSSION

Colorectal cancer (CRC) is increasing its incidence worldwide, particularly in low-income countries but also in developed countries andit already represents the second leading cause of death from cancer (22, 37). Both morphological studies and recent molecular classifications of the CRC have highlighted the relevance of the tumor microenvironment, rekindling

Variables	N	Percent (%)
Gender		
Male	180	53.8
Female	210	46.2
Total	390	100
Age Range		
19 to 40 years	25	6.4
41 to 50 years	46	11.8
51 to 60 years	82	21.0
61 to 70 years	101	25.9
Above 70 years	136	34.9
Total	390	100
Topography		
Right	115	29.5
Left	171	43.8
Rectal	104	26.7
Total	390	100
Histological type		
Adenocarcinoma NOS	360	92.3
Mucinous adenocarcinoma	30	7.7
Total	390	100
Histological grade		
G1 Well differentiated	8	2.1
G2 Moderately differentiated	357	91.5
G3 Poorly differentiated	25	6.4
Total	390	100
T-status		
pTis	4	1.0
pT1	7	1.8
pT2	45	11.5
pT3	278	71.4
pT4a	20	5.1
pT4b	36	9.2
Total	390	100
Tumor budding		
Null	6	1.5
No	278	71.3
Yes	106	27.2
Total	390	100
Perineural invasion	203	52.1
Angiolymphatic invasion	190	48.7
Lymph node metastasis	183	46.9
Distant metastasis	36	9.2

Table 1: Absolute and relative frequency of clinico-pathological characteristics

interest in the "seed and soil" hypothesis of colorectal carcinogenesis (4, 38). Previous morphological studies have examined the stromal tumor relationships in the microenvironment of epithelial cancers (30–36). In our study tumor microenvironment and estimation of the proportion of tumor stroma in colorectal adenocarcinomas, in a middle-income country was evaluated.

In the surgically resected CRA stroma-high cases were detected in 53.3% of the patients, a substantially larger stromal proportion compared to the average (24-29%) in initial studies (6–8, 19, 39), but similar to some more recent studies (5, 13, 15–17) that pointed out a stromal estimate of up to 47%.

The association found in this study between exclusively well-differentiated colorectal adenocarcinomas and low stroma was particular, and not seen in other series (5, 8, 15), indicating that the loss of differentiation was accompanied by stromal expansion. In a way to understand these findings, a pioneering study in the characterization and validation (in vitro and in vivo co-cultures) of the tumor microenvironment in CRC, observed that disordered extracellular matrix, with higher stroma proportion, drove a mesenchymal phenotype towards being similar to poorly differentiated (40).

Our results showed that stroma-rich CRAs are significantly associated with adverse pathological characteristics, such as perineural invasion and lymphatic invasion, similarly to previous findings (15, 20, 41).

Patients with a high stromal proportion more often had a tumor budding compared to stroma - poor tumors. The present findings may indicate a relevant role of tumor stroma in facilitating the differentiation, spread of tumor cells and in the epithelial-mensenchymal transition. This expressive association was also observed in other studies (8,17,20,42), although the complete elucidation of the molecular basis is still needed. A recent study showed that an increase in the composition of the tumor stroma and a reduction in epithelial cellularity were significantly correlated with genetic signatures related to epithelial-mensenchymal transition (12).

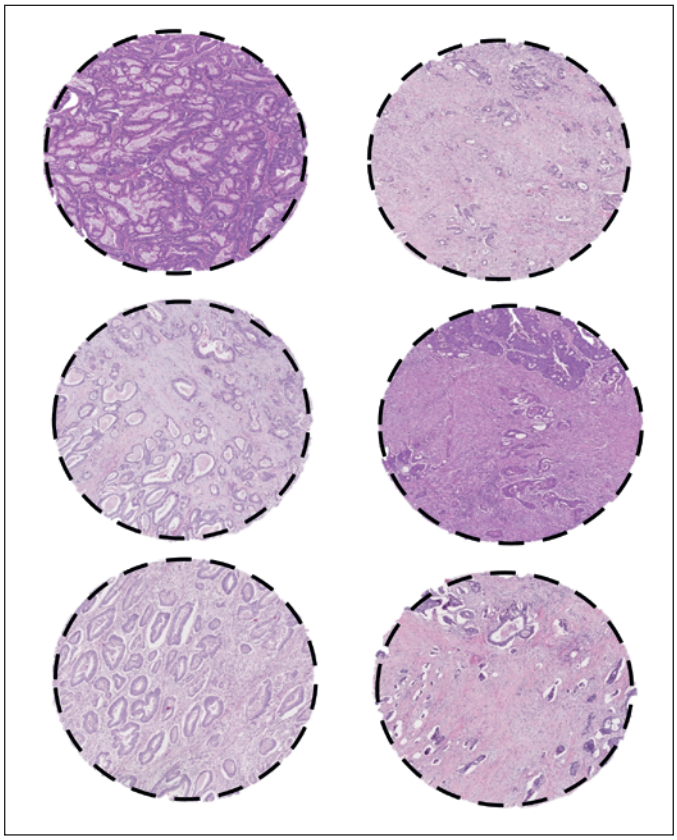

Figure 2. Tumor-stroma proportion.
Left column: Examples of TSP ≤ 50% - poor in stroma (Cases No. 382,391 and 284, top to bottom).
Right column: Examples of TSP > 50% - rich in stroma (Cases No. 249,119 and 73, top to bottom).
Hematoxylin-eosin; magnification ×10.

Variables	TSP		Total	P[1]
	≤ 50%	> 50%		
	N (%)	N (%)	N (%)	
Histological grade				
G1	7 (4.0)	1 (0.5)	8 (2.1)	0.028*
G2	160 (90.9)	191 (91.8)	351 (91.4)	n.s.
G3	9 (5.1)	16 (7.7)	25 (6.5)	
Perineural invasion				
No	117 (66.5)	67 (32.2)	184 (47.9)	<0.001*
Null	0 (0.0)	1 (0.5)	1 (0.3)	n.s.
Yes	59 (33.5)	140 (67.3)	199 (51.8)	
Angiolymphatic invasion				
No	121 (68.8)	77 (37.0)	198 (51.6)	<0.001*
Yes	55 (31.3)	131 (63.0)	186 (48.4)	n.s.
Lymph node metastasis				
No	113 (64.2)	85 (40.9)	198 (51.6)	<0.001*
Null	5 (2.8)	2 (1.0)	7 (1.8)	n.s.
Yes	58 (33.0)	121 (58.2)	179 (46.6)	
Distant metastasis				
No	0 (0.0)	1 (0.5)	1 (0.3)	0.001*
Null	169 (96.0)	178 (85.6)	347 (90.4)	n.s.
Yes	7 (4.0)	29 (13.9)	36 (9.4)	
pT-status				
pT1	10 (5.7)	0 (0.0)	10 (2.6)	<0.001*
pT2	34 (19.3)	11 (5.3)	45 (11.7)	n.s.
pT3	113 (64.2)	160 (76.9)	273 (71.1)	
pT4	19 (10.8)	37 (17.8)	56 (14.6)	
pTis	3 (1.7)	0 (0.0)	3 (0.8)	
Tumor budding				
No	154 (87.5)	124 (59.6)	278 (72.4)	<0.001*
Yes	22 (12.5)	84 (40.4)	106 (27.6)	n.s.
Prognostic Stage				
p- Stage 0	3 (1.7)	0 (0.0)	3 (0.8)	<0.001*
p- Stage I	35 (19.9)	8 (3.8)	43 (11.2)	n.s.
p- Stage II	79 (44.9)	74 (35.6)	153 (39.8)	
p- Stage III	51 (29.0)	95 (45.7)	146 (38.0)	
p- Stage IV	7 (4.0)	29 (13.9)	36 (9.4)	
Null	1 (0.5)	2 (1.0)	3 (0.8)	
Total	**176 (100.0)**	**208 (100.0)**	**384 (100.0)**	

[1] Likelihood ratio test
* Statistically significant (p <0.05)
n.s. – non-significant

Table 2- Clinico-pathological data and their association with the tumor-stroma proportion (TSP)

The depth of invasion through the intestinal wall and the presence of lymph node metastases, which are recognized as the most important independent prognostic factors in CRC, related to survival and risk of recurrence, in addition to indicating the therapeutic approach (22, 43, 44), were significantly related to the percentage of stroma.

All pT1 patients were stroma-low, while 75.6% of the pT2 patients were stroma-low. In the pT3 group, this percentage decreased to 41.4% and in the pT4 group it was 33.9%. Stroma- poor tumors showed less depth of invasion. These results point out that the expansion of the stromal compartment presents itself as a characteristic of aggressive disease, more locally advanced. The stromal proportion observed for the pT3-pT4 status was significantly higher, compared to early-stage tumors (TSP >50% - pT4: 66.1%; pT3: 58.6%; pT2: 24.4%; pT1: 0%; pTis: 0%).

Percent of high stroma in patients with lymph node metastases was 67.6% (121 cases out of 179). The important association found between stromal estimation >50% and positive lymph nodes was also seen in other studies with colorectal cancer (8, 15, 20, 45).

The expansion of the stromal component was also a risk factor independent of the occurrence of distant metastases. Almost all stroma-rich CRC (80.6%) presented distant metastasis when surgicaly resected, an association also observed in a recent study (20). The observation of this association is particularly important in the therapeutic field, since the hypotheses indicate that the interruption of tumor-stroma interactions can inhibit or help eliminate tumor progression and metastasis (19), as well as that the stromal component may contribute to chemoresistance (8).

In recent years, a better understanding of the processes of carcinogenesis have fostered research into new biomarkers with the ultimate aim of promoting more personalized and effective therapy. In the case of CRC, especially for non-metastatic tumors (p-Stage II), which comprise a heterogeneous group with a different outcome (24) the classic histopathological classification is not sufficiantly informative for planning the treatment of these patients (21).

The present study showed a strong association between stromal estimation and prognostic stage of the disease, that was in agreement with previous studies (6, 7, 45). Adenocarcinomas without lymph node metastasis or distant metastases had TSP ≤ 50%, while advanced disease (with lymph node and distant metastases) exhibited TSP > 50% thus demonstrating that stromal expansion in CRA represents a marker of tumor progression, associated with aggressive disease.

No association was found between the tumor-stroma proportion and certain factors, in agreement with results from previous series, such as: male or female sex (5, 7, 8, 13, 41); age groups (8, 13, 41); location of the neoplasm (7, 17, 41) and histological type (13, 15).

The adoption of a morphometric methodological protocol widely used by independent international groups (9, 10, 15–18) for colon and rectal cancer, without new costs, proved to be highly reproducible, with good agreement by different observers confirming its applicability from previous studies (5–7, 9, 19, 46). It is important to highlight that the visual selection of the area analyzed by the pathologist is vital for the current approach in the CRA (47).

CONCLUSIONS

The description of the microscopic features, through hematoxylin and eosin staining, selected from the tumor microenvironment, alone or in combination, provides considerable information in colonic and rectal cancer. Tumor-stroma proportion is associated with all known histological characteristics, which reflect on an adverse clinical prognosis and guide the management of patients. The correlation between high stroma and advanced disease indicates that a high proportion of stromal tumor tissue can promote host tissue invasion and tumor aggressiveness, reflecting the defining contribution of the stromal compartment. The tumor-stroma proportion expresses the stage of the disease and can

potentially complement the current staging system, even allowing a morphological correlation with the development of molecular classifications, in a precise, reproducible way and with applicability in routine diagnostics.

Acknowledgements

Authors would like to thank Alexandre Rolim da Paz, pathologist of the Pathology Laboratory of the University Hospital Lauro Wanderley and the Pathology Laboratory of the Cancer Hospital Napoleão Laureano.

Declaration of Interests

Authors declare no conflicts of interest.

REFERENCES

1 Colangelo T, Polcaro G, Muccillo L, D'Agostino G, Rosato V, Ziccardi P, et al. Friend or foe? The tumour microenvironment dilemma in colorectal cancer. Biochim Biophys Acta Rev Cancer. 2017 Jan;1867(1):1–18.

2 Colvin H, Mori M. Colorectal cancer: Back to the stroma--the real villain in colorectal cancer? Nat Rev Gastroenterol Hepatol. 2015 May;12(5):256–8.

3 Huet E, Jaroz C, Nguyen HQ, Belkacemi Y, Taille A, Stavrinides V, et al. Stroma in normal and cancer wound healing. FEBS J. 2019 Aug;286(15):2909–20.

4 Jakubowska K, Kisielewski W, Kańczuga-Koda L, Koda M, Famulski W. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. Oncol Lett. 2017 Dec;14(6):6421–32.

5 Scheer R, Baidoshvili A, Zoidze S, Elferink MAG, Berkel AEM, Klaase JM, et al. Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study. World J Gastrointest Oncol. 2017 Dec 15;9(12):466–74.

6 Mesker WE, Junggeburt JMC, Szuhai K, de Heer P, Morreau H, Tanke HJ, et al. The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. Cell Oncol. 2007;29(5):387–98.

7 West NP, Dattani M, McShane P, Hutchins G, Grabsch J, Mueller W, et al. The proportion of tumour cells is an independent predictor for survival in colorectal cancer patients. Br J Cancer. 2010 May 11;102(10):1519–23.

8 Park JH, Richards CH, McMillan DC, Horgan PG, Roxburgh CSD. The relationship between tumour stroma percentage, the tumour microenvironment and survival in patients with primary operable colorectal cancer. Ann Oncol. 2014 Mar;25(3):644–51.

9 De Wever O, Mareel M. Role of tissue stroma in cancer cell invasion. J Pathol. 2003 Jul;200(4):429–47.

10 Sandberg TP, Stuart MPME, Oosting J, Tollenaar RAEM, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC Cancer. 2019 Mar 29;19(1):284.

11 Ribeiro Franco PI, Rodrigues AP, de Menezes LB, Pacheco Miguel M. Tumor microenvironment components: Allies of cancer progression. Pathol Res Pract. 2020 Jan;216(1):152729.

12 McCorry AM, Loughrey MB, Longley DB, Lawler M, Dunne PD. Epithelial-to-mesenchymal transition signature assessment in colorectal cancer quantifies tumour stromal content rather than true transition. J Pathol. 2018 Dec;246(4):422–6.

13 van Pelt GW, Hansen TF, Bastiaannet E, Frifeldt SK, van Krieken JHJ, AEM Tollenaar R, et al. Stroma-High Lymph Node Involvement Predicts Poor Survival More Accurately for Patients with Stage III Colon Cancer. J Med Surg Pathol [Internet]. 2016 [cited 2020 Oct 27];01(02). Available from: https://www.omicsonline.org/open-access/stromahigh-lymph-node-involvement-predicts-poor-survival-more-accuratelyfor-patients-with-stage-iii-colon-cancer-jmsp-1000116.php?aid=72496

14 Ao T, Kajiwara Y, Yonemura K, Shinto E, Mochizuki S, Okamoto K, et al. Morphological consistency of desmoplastic reactions between the primary colorectal cancer lesion and associated metastatic lesions. Virchows Arch. 2020 Jul;477(1):47–55.

15 Hansen TF, Kjær-Frifeldt S, Lindebjerg J, Rafaelsen SR, Jensen LH, Jakobsen A, et al. Tumor-stroma ratio predicts recurrence in patients with colon cancer treated with neoadjuvant chemotherapy. Acta Oncol. 2018 Apr;57(4):528–33.

16 Sandberg TP, Oosting J, van Pelt GW, Mesker WE, Tollenaar RAEM, Morreau H. Erratum: Molecular profiling of colorectal tumors stratified by the histological tumor-stroma ratio - Increased expression of galectin-1 in tumors with high stromal content. Oncotarget. 2019 22;10(24):2416.

17 van Wyk HC, Roseweir A, Alexander P, Park JH, Horgan PG, McMillan DC, et al. The Relationship Between Tumor Budding, Tumor Microenvironment, and Survival in Patients with Primary Operable Colorectal Cancer. Ann Surg Oncol. 2019 Dec;26(13):4397–404.

18 Abstracts: 31st European Congress of Pathology. Virchows Arch. 2019 Sep 1;475(S1):1–436.

19 Huijbers A, Tollenaar R a. EM, v Pelt GW, Zeestraten ECM, Dutton S, McConkey CC, et al. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Ann Oncol. 2013 Jan;24(1):179–85.

20 Martin B, Banner BM, Schäfer E-M, Mayr P, Anthuber M, Schenkirsch G, et al. Tumor proportion in colon cancer: results from a semiautomatic image analysis approach. Virchows Arch. 2020 Aug;477(2):185–93.

21 Geessink OGF, Baidoshvili A, Klaase JM, Ehteshami Bejnordi B, Litjens GJS, van Pelt GW, et al. Computer aided quantification of intratumoral stroma yields an independent prognosticator in rectal cancer. Cell Oncol (Dordr). 2019 Jun;42(3):331–41.

22 Nagtegaal ID, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, et al. The 2019 WHO classification of tumours of the digestive system. Histopathology. 2020;76(2):182–8.

23 Amin MB, American Joint Committee on Cancer, American Cancer Society, editors. AJCC cancer staging manual. Eight edition / editor-in-chief, Mahul B. Amin, MD, FCAP ; editors, Stephen B. Edge, MD, FACS [and 16 others] ; Donna M. Gress, RHIT, CTR-Technical editor ; Laura R. Meyer, CAPM-Managing editor. Chicago IL: American Joint Committee on Cancer, Springer; 2017. 1024 p.

24 Schmoll HJ, Van Cutsem E, Stein A, Valentini V, Glimelius B, Haustermans K, et al. ESMO Consensus Guidelines for management of patients with colon and rectal cancer. a personalized approach to clinical decision making. Ann Oncol. 2012 Oct;23(10):2479–516.

25 Zunder SM, Gelderblom H, Tollenaar RA, Mesker WE. The significance of stromal collagen organization in cancer tissue: An in-depth discussion of literature. Critical Reviews in Oncology/Hematology. 2020 Jul 1;151:102907.

26 Gasparini B, Valadão M, Miranda-Filho A, Silva CMFP da. [Analysis of the age-period-cohort effect on mortality from colorectal cancer in Rio de Janeiro State, Brazil, from 1980 to 2014]. Cad Saude Publica. 2018 12;34(3):e00038017.

27 van Pelt GW, Krol JA, Lips IM, Peters FP, van Klaveren D, Boonstra JJ, et al. The value of tumor-stroma ratio as predictor of pathologic response after neoadjuvant chemoradiotherapy in esophageal cancer. Clinical and Translational Radiation Oncology. 2020 Jan 1;20:39–44.

28 van Pelt GW, Kjær-Frifeldt S, van Krieken JHJM, Al Dieri R, Morreau H, Tollenaar R a. EM, et al. Scoring the tumor-stroma ratio in colon cancer: procedure and recommendations. Virchows Arch. 2018 Oct;473(4):405–12.

29 Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics. 1977 Jun;33(2):363–74.

30 Ahn S, Cho J, Sung J, Lee JE, Nam SJ, Kim K-M, et al. The prognostic significance of tumor-associated stroma in invasive breast carcinoma. Tumour Biol. 2012 Oct;33(5):1573–80.

31 de Kruijf EM, van Nes JGH, van de Velde CJH, Putter H, Smit VTHBM, Liefers GJ, et al. Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple-negative carcinoma patients. Breast Cancer Res Treat. 2011 Feb;125(3):687–96.

32 Dekker TJA, van de Velde CJH, van Pelt GW, Kroep JR, Julien J-P, Smit VTHBM, et al. Prognostic significance of the tumor-stroma ratio: validation study in node-negative premenopausal breast cancer patients from the EORTC perioperative chemotherapy (POP) trial (10854). Breast Cancer Res Treat. 2013 Jun;139(2):371–9.

33 Zhang T, Xu J, Shen H, Dong W, Ni Y, Du J. Tumor-stroma ratio is an independent predictor for survival in NSCLC. Int J Clin Exp Pathol. 2015;8(9):11348–55.

34 Liu J, Liu J, Li J, Chen Y, Guan X, Wu X, et al. Tumor-stroma ratio is an independent predictor for survival in early cervical carcinoma. Gynecol Oncol. 2014 Jan;132(1):81–6.

35 Courrech Staal EFW, Wouters MWJM, van Sandick JW, Takkenberg MM, Smit VTHBM, Junggeburt JMC, et al. The stromal part of adenocarcinomas of the oesophagus: does it conceal targets for therapy? Eur J Cancer. 2010 Mar;46(4):720–8.

36 Pietras K, Ostman A. Hallmarks of cancer: interactions with the tumor stroma. Exp Cell Res. 2010 May 1;316(8):1324–31.

37 Siegel RL, Miller KD, Sauer AG, Fedewa SA, Butterly LF, Anderson JC, et al. Colorectal cancer statistics, 2020. CA: A Cancer Journal for Clinicians. 2020;70(3):145–64.

38 Hynes SO, Coleman HG, Kelly PJ, Irwin S, O'Neill RF, Gray RT, et al. Back to the future: routine morphological assessment of the tumour microenvironment is prognostic in stage II/III colon cancer in a large population-based study. Histopathology. 2017 Jul;71(1):12–26.

39 Mesker WE, Liefers G-J, Junggeburt JMC, van Pelt GW, Alberici P, Kuppen PJK, et al. Presence of a high amount of stroma and downregulation of SMAD4 predict for worse survival for stage I-II colon cancer patients. Cell Oncol. 2009;31(3):169–78.

40 Devarasetty M, Dominijanni A, Herberg S, Shelkey E, Skardal A, Soker S. Simulating the human colorectal cancer microenvironment in 3D tumor-stroma co-cultures in vitro and in vivo. Sci Rep [Internet]. 2020 Jun 17 [cited 2021 Jun 8];10. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7300090/

41 Eriksen AC, Sørensen FB, Lindebjerg J, Hager H, de Pont Christensen R, Kjær-Frifeldt S, et al. The prognostic value of tumour stroma ratio and tumour budding in stage II colon cancer. A nationwide population-based study. Int J Colorectal Dis. 2018;33(8):1115–24.

42 Ueno H, Jones AM, Wilkinson KH, Jass JR, Talbot IC. Histological categorisation of fibrotic cancer stroma in advanced rectal cancer. Gut. 2004 Apr;53(4):581–6.

43 Pereira Júnior T, Alves AJ da C, Nogueira AMMF. Câncer colorretal: análise anatomopatológica de 476 colectomias consecutivas em Belo Horizonte (MG). J Bras Patol Med Lab. 2005 Jun;41(3):175–84.

44 Volpato MG, Koch K de S. Fatores de mau prognóstico nas peças operatórias de pacientes submetidos ao tratamento cirúrgico do câncer colorretal. Rev bras coloproctol. 2008 Dec;28(4):414–24.

45 Wu J, Liang C, Chen M, Su W. Association between tumor-stroma ratio and prognosis in solid tumor patients: a systematic review and meta-analysis. Oncotarget. 2016 Oct 18;7(42):68954–65.

46 Souza da Silva RM, Queiroga EM, Paz AR, Neves FFP, Cunha KS, Dias EP. Standardized Assessment of the Tumor-Stroma Ratio in Colorectal Cancer: Interobserver Validation and Reproducibility of a Potential Prognostic Factor. Clin MedInsightsPathol. 2021 Jan 1;14:2632010X21989686.

47 Micke P, Strell C, Mattsson J, Martín-Bernabé A, Brunnström H, Huvila J, et al. The prognostic impact of the tumour stroma fraction: A machine learning-based analysis in 16 human solid tumour types. EBioMedicine 2021 Mar 1; 65.